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BY

M. L. HOLBROOK, M. D.

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THE EFFECT OF DILUTE SOLUTIONS OF CHROMIC ACID AND ACID URINE UPON THE RED BLOOD CORPUSCLES OF MAN.

M. L. Holbrook, M. D.

When Louis Elsberg, in 1879,* announced that by the addition of a 40 to 50 per cent. saturated solution of bichromate of potash within a few hours a reticulated structure becomes visible in the red blood corpuscles, his assertions met with grave doubts. To those who repeated Elsberg's experiments, and either could not see the reticulum or when seen considered it to be artificial and produced by the chemical reagent used, Elsberg's reply was that a solution of bichromate of potash was used successfully for the preservation of the most delicate tissues of the animal body, such as brain, spinal cord, etc. Nevertheless it seemed to me desirable, if possible, to bring this structure to view by reagents which I think cannot be charged with producing artificial appearances. For this reason I made a series of experiments with blood from a large number of persons which I kept in a moist chamber in its own serum. After several days the reticulated structure became visible in every fully developed blood corpuscle. A full account of these experiments was read before this Society in 1892 and appears in the Proceedings for that year. There I said that by this method the hæmoglobin was gradually extracted by the blood serum, and the reticulum, be-being identical with that of protoplasm in general, becomes visible. I also stated that the reticulum is not very pronounced, and that it requires a good immersion lens as well as a thoroughly trained eye to see it satisfactorily.

Since then I have sought for other methods, and first of all tried the effects of weak solutions of chromic acid upon the corpuscles.

*Annals of the New York Academy of Science, vol. i.

Such solutions, as is well known, are largely used in laboratories for histological and pathological research, and are considered most reliable for hardening and preserving tissues of all kinds, either normal or morbid.

My way of proceeding was first to oil a narrow space around the margin of one side of a thin covering glass; next to prick the palmer aspect of the left thumb with a fine needle under aseptic precautions. A droplet of blood of the size of a pin-head is thus obtained and transferred to the slide. Before applying the cover, a small drop of the chromic acid is applied over the blood by means of a camel's-hair brush or a dropper and at once covered with the oiled cover-glass, the oiled edges, coming in contact with the slide, forming a frame around the blood. By this manner the drop of blood is excluded from the influence of the air and prevented from drying; it remains fit for observation for several days. Prepared thus, the changes in the red blood corpuscles could be observed with the highest powers of the microscope at my disposal. I used Tolles' 16th-inch water immersion lens, amplifying 1,200 diameters. At first a half of one per cent. of chromic acid solution was tried; the changes could be observed at once, after it was ready for study, and remained unchanged so long as the specimen was kept. On the 8th day after mounting everything appeared the same as at first. The effect of this solution is the immediate extraction of the hæmoglobin and a tetanic contraction of the reticulum of living matter. In many blood corpuscles there appeared a double contour with granules adhering to it, whereas, either in the center or margin, there was a lump of a yellow substance either homogeneous, irregularly outlined, or made up of a varying number of granules. The space between the outer shell and the lump described was usually light and apparently devoid of any structure. Occasionally a corpuscle would show an indistinct reticulum and a few large threads traversing the light space between the contracted mass and the inner contour. The next experiment was made with a one-third per cent. solution of chromic acid. The result was very much like that obtained with a half per cent. solution, and needs no further description. From what I saw in these specimens I became convinced that these solutions were too strong, causing sudden and intense contraction, termed tetanic by the physiologists, the lump or mass located in various parts of the corpuscles being only the reticulum contracted by the spasm, so to say, as soon as it

came in contact with the acid. This fact, however, seems to be one more proof of the reticulum being made up of living matter, for it seems to me it is only this matter which can contract in the way described. A fourth of a one per cent. solution was now used and found to be more satisfactory in its action. Some of the red blood corpuscles showed a distinct reticular structure identical with that described by Elsberg in 1879 and by myself in 1892; such corpuscles, however, were in a minority, whereas the largest number showed the corpuscles enlarged and traversed by an extremely faint reticulum holding large colorless meshes—a condition which is usually turned hydropic. Other corpuscles showed figures of contraction similar to those observed after the addition of stronger solutions. I now resorted to a one-fifth, one-sixth, one-seventh, and one-eighth per cent. solution, but the one-fifth per cent. proved the best of all in my hands and better than the one-fourth per cent. solution.

The reticulum appeared in a large number of the corpuscles, although others appeared pale and hydropic. This solution is sufficiently strong to extract the hæmoglobin almost immediately, at the same time rendering the granules and threads of living matter yellowish and plainly visible. In the latter specimens we see a shell bordering the corpuscles, making a double contour. From this shell arise all around a number of conical spokes, interosculating with adjacent granules, the intersection of the reticulum.

Occasionally we observe in the interior somewhat larger lumps, obviously caused by a contraction of a portion of the reticulum as seen in stronger solutions. After the application of a one-sixth of one per cent. solution those with a reticulum appeared to be few in number, whereas the majority of corpuscles had become hydropic, exhibiting only traces of this structure. Any one desirous of repeating these interesting experiments is advised to prepare a one-fifth per cent. chromic acid solution and add it to the droplet of fresh blood, as before described. Although the result is gratifying, still many may think the same objection raised to the use of the solution of bichromate of potash holds good for solutions of chromic acid. Conservative persons will not be convinced by either of these reagents unless substantiated by others, and for this reason I resolved to try another agent which I believe no one will suspect to be a poison to a red blood corpuscle, and this reagent is fresh normal acid urine.

Part II.

In C. Heitzmann's laboratory, where I have pursued my studies for eighteen years, observations had been made that in certain abnormal urines sent for examination the red blood corpuscles assume, if viewed with medium powers of the microscope, a granular appearance; some of the corpuscles even exhibited broad offshoots, so-called pseudopodia, similar to those seen in colorless blood corpuscles. The resemblance between these two kinds of corpuscles was frequently found so great that it was only by their size that they were distinguished.

Faber* observed in the urine of a patient with Bright's disease colored blood corpuscle of a great variety of different shapes, some of which showed him phenomena of contractility and amæboid movement very similar to those seen in colorless corpuscles.

These observations have induced me to study the influence of urine upon the red blood corpuscles. For this purpose I oiled the edges of a covering glass as previously described, took a droplet of blood on the slide, my own as well as others, and at once mixed it with a drop of urine. Immediately after mounting peculiar changes could be seen. Most of the corpuscles appeared beset at their periphery with a large number of delicate thorn-like projections, which in the top view appeared like very delicate dots besetting the surface of the disk. Soon afterward many corpuscles showed delicate knobs. At the same time the contours of the corpuscles became irregular and indentated with numerous notches. Four hours afterward most of the thorny projections had disappeared. A few of the knobs had increased in size, almost reaching one-fourth of the diameter of the main body. The knob at first showed a distinct attachment to the main body, which was correspondingly reduced in size. A few of the knobs apparently had become detached and floated in the neighborhood of the corpuscle. Several knobs of large size being attached to the main body, the impression was conveyed that the red blood corpuscles had split up into a number of smaller pieces. Some of the corpuscles flattened out and became slightly enlarged, exhibiting a large central vacuole or a varying number of small vacuoles scattered throughout its

* Uber die rothen Blutkorperchen, Archiv. der Heilkunde, 1873, vol. xiv, pp. 481–511.

body. In that the reaction was very similar to that of dilute solution of bichromate of potash, I now remembered that the vessel shortly before using had been cleaned with a strong solution of bichloride of mercury, no doubt affecting the urine. This observation therefore, though interesting, I considered as of little account.

In the next experiment I was careful to obtain absolutely pure urine of an acid reaction. A small drop of it was added to the blood as soon as it was put on the slide, and at once covered with the cover-glass previously prepared by oiling the border of the margin as before described. Only a little urine can be used, for it fills the space and runs over the oil, thus leaving unprotected places for the entrance of air. Again the immediate effect was the appearances of crenations at the periphery of the corpuscles and dots upon the surface, simulating a reticulum and deceiving the inexperienced. Twenty-four hours later the changes were not pronounced. In the next few days the number of micrococci and spores of mildew had considerably increased and the number of red blood corpuscles had greatly diminished; the few left were either ghostlike or showed only a pale reticulum. The same result occurred in all subsequent experiments of this description. With the rapidly increasing number of leptothrix threads, the blood corpuscles disappeared. Notwithstanding the failure of this experiment for the purpose intended, I advise others to make it on account of its beauty. The urine seems to dissolve the serum and all its contents, including fibrin, which always comes to view in using the chromic acid and gives a dirty appearance to the specimens. Each corpuscle separates from the other and can be studied by itself, and the curious crenations are different from any I have seen. The corpuscles lose their concavity, and the thorny projections may be seen from their sides as well as margins. There is considerable variety of forms.

I now changed my method, and let two or three drops of blood from the forefinger, cut with the point of a sharp lancet, run into a one-ounce bottle filled with fresh urine, so as to have a larger quantity to act on the blood than the small amount which could be placed on the slide. The bottle was allowed to rest twenty-four hours, when the upper part was decanted, only the sediment transferred into a small dish, and a drop transferred to the slide as before. The corpuscles now under the microscope showed a surprising view. Almost all of them exhibited the reticular structure quite perfectly; only in the small-sized corpuscles, the hæmatoblasts of Hayem, were they

not seen. After having obtained this highly gratifying result I made several experiments to discover what kind of urine was best for bringing out the reticular structure. Pale urine of a specific gravity of less than 1,020 and of only a slightly acid or neutral reaction was found unsuitable. In some corpuscles the reticulum was faint and pale and not well pronounced. Even the addition of a drop of a 50 per cent. solution of chromic acid to the ounce of urine did not prevent it from becoming alkaline after forty-eight hours, and as soon as the crystals of triple phosphate, micrococci, and bacilli appeared the corpuscles, at least a majority, had become pale, hydropic, and soon afterward completely disappeared.

It is only a dark amber-colored, distinctly acid urine of a specific gravity of at least 1,022, preferably 1,024, which, being mixed with fresh-drawn blood and kept at rest in a cool place, will produce the desired effect. From twenty to twenty-four hours afterward the urine has dissolved out, the hæmoglobin filling the meshes of the reticulum and the liquid appearing to the naked eye of a distinctly bloody tint. The liquid being carefully decanted, the sediment may be utilized for the demonstrations of the reticulum with high powers of the microscope with, I think, almost universally good results.

Since fresh acid urine is an excellent medium for preserving temporarily the structure of the colorless blood corpuscles as well as the pus corpuscles, both of which remain amæboid as long as the urine is sufficiently warm, I do not see how any one can distrust this reagent, as was the case with solutions of bichromate of potash and possibly with solutions of chromic acid. In fact, I cannot conceive of a more simple and trustworthy method than the one described above.

The question now arises: How can we preserve the reticulum for demonstration months or years afterward? This may be done by the addition of chromic acid solution to the sediment collected in a small dish as follows: To half a drachm of such sediment two large drops of a 50 per cent. solution of chromic acid should be added. The dish must be kept covered with a large glass dish for five or six days, in order to give the chromic acid a chance to fix the reticulum. After this chemically pure glycerine, preferably that from Merck of Darmstadt, Germany, should be added to the sediment, which is now left uncovered for a few days to allow the evaporation of the water. Such a glycerine specimen is ready for

use at any time. Large quantities of blood, as is often seen in highly acid urine in cases of pelvic hemorrhage due to pelvic calculi, after the treatment with the chromic acid solution, may be placed in a bottle, to which add 50 per cent alcohol in order to prevent the growth of mildew; such a preparation is ready for demonstrations at any time with or without the addition of glycerine. In C. Heitzmann's laboratory urine sediments have been kept in the above-described manner in bottles with glass stoppers for fifteen or more years without changing the reticulated structure of the corpuscles.

Acid urine is probably the best agent to bring out the structure of the red blood corpuscles yet found.

It is not objectionable, for it does not cause artificial features.

Any one who has seen the reticulum of living matter in colorless and pus corpuscles, a feature nowadays reproduced by photo-micrography, has the means of satisfying himself concerning this structure.

I recommend those who still doubt, as I know many do, to read Stricker's Arbeiten aus dem Institute für Allegemeine und experimentelle Pathologie, Wiener Universität, 1890, where this author gives a picture of the white blood corpuscle of a newt (Proteus) by the heliogravure process with a power of 2,500, with the aid of the electric microscope.